7		_
_	_	=
C	ľ	)
•	-	
L	l	-

					1/54		<del></del>		
Activity	U/g IB	0	0	0	40.00	0	105.26	15.65	48.70
550 mM	L- Arg	0	+	0	+	0	+	0	+
440 m.M	Sucrose	0	+	+	0	+	0	0	+
2.2 m.M	CaCl <sub>2</sub>	+	0	+	0	0	+	0	-1-
2.2 mM	MgCl <sub>2</sub>	+	0	+	0	0	+	0	+
1.1 mM	EDTA	0	+	. 0	+	+	0	+	0
550 mM	GndHCI	+	+	0	0	0	0	+	+
0.055%	PEG 3350	0	+	0	+	0	+	0	+
MM	Š	0.44	0.44	£	<del></del>	0.44	0.44	=	11
mm	NaCl	10.56	10.56	264	264	10.56	10.56	264	264
0.3 mM	LM	+	0	0	+	+	<b>^</b> 0	0	+
0.1 MM	GSSG	+	+	+	+	+	4-	+	+
1 mM	GSH	+	+	+	+	+	+	+	+
	#	2 (55 mM MES pH 6.5)	3 (55 mM MES pH 6.5)	#5 (55 mM MES pH 6.5)	#8 (55 mM MES pH 6.5)	#10 (55 mM MES pH 8.2)	#11 (55 mM Tris pH 8.2)	#13 (55 mM Tris pH 8.2)	#16 (55 mM Tris pH 8.2)

WO 2005/089102

#### **SUBSTITUTE SHEET (RULE 26)**

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#### ELution of MBP-ST3Gal III from Amylose column

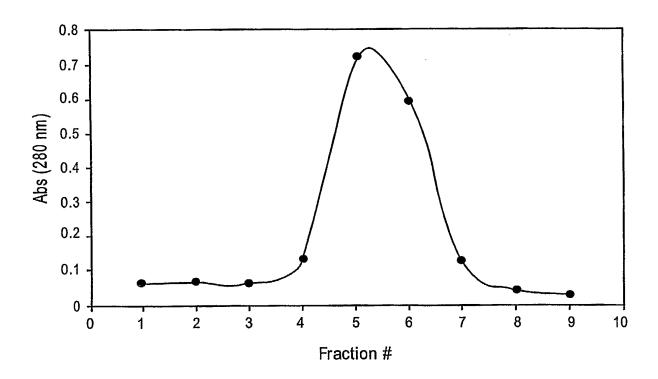


FIG. 2

#### ST3 Gal III activities of the Amylose purified refolded MBP-ST3Gal III fractions

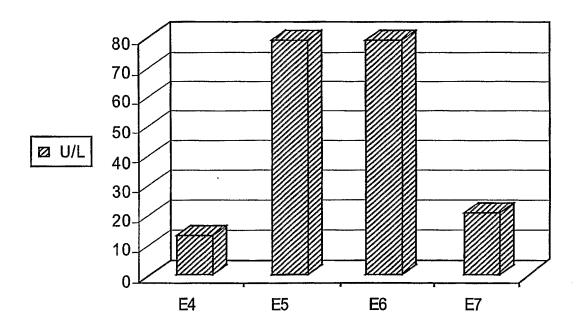


FIG. 3

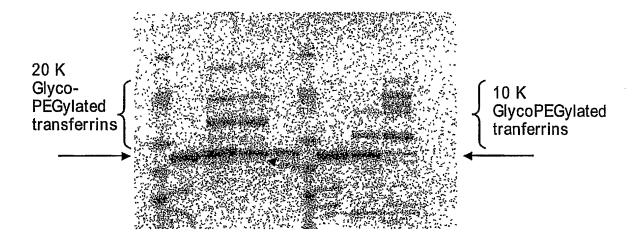
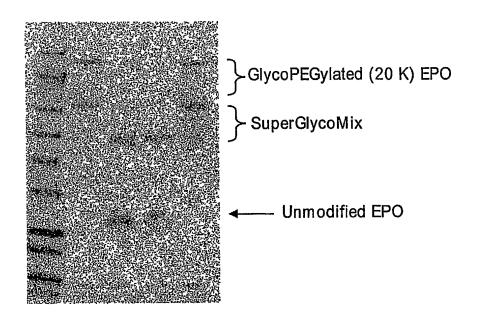


FIG. 4



GlycoPEGylation (20 K) of EPO

FIG. 5

P27115			PPQTWDGYDP: 440		FIG	G. 6	
/usr/t	IVTFQ	FRGRRVHLAI	PPIWEGYDP				
	420	430	440				
ماسد (ماسد	·	370		390		410	420
P27115				EVRVQYTGRD:			
/usr/t	LARVY	GAPQLQVEKV	RINDRKELGE	EVRVQYTGRD:	SFKAFAKALG		
	360	370	380	390	400	410	
P27115	KGRAC	VRPEISRTMI 310	FGRKGVSHGÇ 320	OFFDQHLKFII 330	340	QLDLSYLQQE 350	AYDRDI 360
	.::::		::::::::::		:::::::	:::::::::::::::::::::::::::::::::::::::	:::::
/usr/t	300 : OGRACI	310 IRPEISRIMI		330 OffDQHLKFI			AYDRDF
	200					350	
P27115	SAWNDI	NGKEQMVDSS 250	KPELLYRTDE 260	FPGLGWLLL <i>i</i> 270	AELWAELEPK 280	WPKAFWDDWM 290	RRPEQR 300
	::::::	::::::::::		:::::::::::	::::::::	:::::::::	::::::
/22 mar /4	240	250	260 ברוייסעד ד עסקיים	270 FPGLGWLLL	280 aetwaete:pki	290 мркағырдым	RRPFAR
		190					230
P27115	FQGYYF		GQIFHNENYE 200	PAAVVVEDDLE 210	VAPDFFEYF 220	QATYPLLKAD 230	PSLWCV 240
	::::::		::.::	:::::::::	::::::::	. : : : : : : : :	:::::
	180 EYYYK	190 CTARHYRWAT		210 PAAVVVEDDLE		230 RATYPLLKAD	PSLWCV
					·		
P27115	RRCLDK	ILHYRPSAE 130	LFPIIVSQDC 140	GHEETAQVIA 150	SYGSAVTHII 160	ROPDLSNIAV 170	QPDHRK 180
	::::::	:::::::::	:::::::::	:::::::::	:::::::::::		:::::
	120 PDCT DK	130	140	150 GHEETAQAIA		170 ROPDISSTAVI	PDHRK
		70	ου				1.20
P27115	AEVELE	RQRGLLQQII 70		WKVPTAAPPA 90	QPHVPVTPPI 100	PAVIPILVIA( 110	CDRSTV 120
	::::::	::::::::	. :: :::	.:::::::	::.:::::	::::::::::	:::::
/** === /±	ייני דיניני אנדי איני	70	~ ~	90 GRVPTAAPPA	100 Oddydydyda'i	110 2007/1077/20	TDRSTT1
		10	20	30	40	50	00
P27115	MLKKQS		LEVAWNALLL	LFFWIRPVPS	RLPSDNALDI	DPASLTREVI	RLAQD 60
	::::::	:::::::::	:::::::::	::::::::	: :: .:::	:::::::::::	:::::
/ /1-	N 4T T 2T 2 ( ) ( )	10	20	30 lffwirpapg	40	50 דע איש מיים אינורניי	60 COA TET

gqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg avipilviacdrstvrrsldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa  ${
m apqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn^*}$ 

GnT1 Cys121Ser mutant

sagottcaaggotttcgccaaggototgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt Geggtgatteceatectggteategeetgtgaeegeageactgtteggege**tet**etagaeaagetgetgeattateggeeeteggt coggoagcogacotgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgccactaccg cctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctga ctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggtggaggatgacctggaggtggccccggacttctt accagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacggt scagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggaggggggtatgatcctagctggaattag

## <u>ال</u>

avipilviacdrstvrrdldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa .gqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae lepkwpk afwddwmr peqrqgrac ir peisrtmt fgrkgv shgqffdqhlk fiklnqqfvhftqldl sylqreaydrd flarvyg ${
m apqlqvekvrt}$ ndrkel ${
m gevrvqyt}$ grdsfkafakal ${
m gvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn}^*$ 

GnT1 Cys121Asp

gccetgagateteaagaacgatgacetttggccgcaagggtgtgagccacgggcagttetttgaccagcaceteaagtttateaagetg cagottcaaggotttcgccaaggototgggtgtcatggatgaccttaagtcggggggttccgagagotggctaccgggggtattgtcacctt aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacgg gctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggtggaggatgacctggaggtggccccggacttct Geggtgatteceateetggteategeetgtgaeegeageactgtteggegegegatetagaeaagetgetgeattateggeeeteggetg atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc scagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggaggggtatgatcctagctggaattag

## FG. 8

avipilviacdrstvrrtldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwal  ${
m gqvfrqfr}$ paavvveddlevapdffeyfratypllkadpslwcvsawndngkeq ${
m mvdasrpellyrtdffpglgwlllaelwael}$  ${
m apqlqvekvrt}$ ndrkel ${
m gevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn}^*$ 

cagetteaaggetttegecaaggetetgggtgteatggatgaecttaagtegggggtteegagagetggetaeegggggattgteaeett gecetgagateteaagaacgatgacetttggeegeaagggtgtgageeaegggeagttetttgaeeageaeeteaagtttateaagetg agetettececcateategttagecaggaetgegggeacgaggagagaeggeccaggecategeetectaeggeageggggggteaegeae gctgggcgctgggccaggtcttccggcagtttcgcttcccgcggccgtggtggtggtggaggatgacctggaggtggccccggacttct aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgccgcgtctacgg Geggtgatteceateetggteategeetgtgacegeageactgtteggegeaetetagaeaagetgetgeattateggeeeteggetg atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc tegagtaetttegggecaectateegetgetgaaggeegaeceeteetgtggtgegteteggeetggaatgaeaaeggeaaggagge ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggaggggtatgatcctagctggaattag

### FG. 9

avipilviacdrstvrraldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae  $apqlqvekvrtndrkel gevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn^{\ast}$ 

GnT1 Cys121Ala

gecetgagateteaagaacgatgacetttggeegeaagggtgtgageeaegggeagttetttgaeeageaeeteaagtttateaagetg cagettcaaggetttegecaaggetetgggtgteatggatgaecttaagtegggggtteegagagetggetaeegggggattgteaeett aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgccgcgtctacgg getgggegetgggecaggtetteeggeagtttegetteeeegeggeegtggtggtggtggaggatgaeetggaggtggeeeeggaettet atccggcagcccgacctgagcagcattgcggtgccggcggaccaccgcaagttccaggggctactacaagatcgcgcgccactacc tcgagtactttcgggccacctatccgctgctgaaggccgaccctcctgtggtgcgtctcggcctggaatgacaacggcaaggagc ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggaggggctatgatcctagctggaattag

## FIG. 1

algqv frq frfp a avvved dlev apd ffey fratypllk adpslwcvs awn dng keq mvd as rpellyrt dffpglgwlllaelwa $a vipilvia c dr stvr {\color{red}{\bf a}} {\color{black}{\bf h}} l dk l l hyrpsa elfpiivs q dcgheeta qaia sygsav thir q p dlssiav p p dhrk f q gyykiar hyrw$ elepkwpk af wddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvy ${
m gapqlq}$ vekvr ${
m tndr}$ kel ${
m gevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwe<math>{
m gydpswn}^*$ 

GnT1 Arg120Ala, Cys121H

gecetgagateteaagaacgatgacetttggeegeaagggtgtgageeacgggeagttetttgaceageaceteaagtttateaagetg cagcticaaggctitcgccaaggctctgggtgtcatggatgaccttaagtcggggggttccgagagctggctaccgggggtattgtcacctt agetettececateategttagecaggaetgegggeacgaggagagaeggeceaggecategeettectaeggeageggeggteaegeae aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgccgcgtctacgg Geggtgatteceatectggteategeetgtgacegeageactgttegg**geeeae**ctagaeaagetgetgeattateggeeeteggetg getgggcgctgggccaggtcttccggcagtttcgcttcccgcggccgtggtggtggtggaggatgacctggaggtggccccggacttct atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc ccagttcccgggccgccgtgtccacctggcgccccaccgacgtgggagggggggtatgatcctagctggaattag

#### 五 5. 4.

Rat Liver ST3Gal III amino acid sequence:

**ARIREFVPPFGIKGQDNLIKAILSVTKEYRLTPALDSLHCRRCIIVGNGGVLANKSLGS** GFLLKLDSKLPAELATKYANFSEGACKPGYASAMMTAIFPRFSKPAPMFLDDSFRKW RIDDYDIVIRLNSAPVKGFEKDVGSKTTLRITYPEGAMQRPEQYERDSLFVLAGFKW <u>MGLLVFVRNILLALCLFLVLGFLYYSAW</u>KLHLLQWEDSNSLILSLDSAGQTLGTEYDRL GLMGRGNIPTLGSVAVTIMALDGCDEVAVAGFGYDMNTPNAPLHYYETVRMAAIKE QDFKWLKYIVYKERVSASDGFWKSVATRVPKEPPEIRILNPYFIQEAAFTLIGLPFNN SWTHNIQREKEFLRKLVKARVITDLSSGI

FIG. 1,

13/54
Full length UDP-N-acetylgalactosaminyltransferase 2 (GalNAcT2)
... nucleic acid and amino acid sequences

Amino acid sequence Met Arg Arg Arg Ser Arg Met Leu Leu Cys Phe Ala Phe Leu Trp Val Leu Gly Ile Ala Tyr Tyr Met Tyr Ser Gly Gly Gly Ser Ala Leu Ala 20 25 30 Gly Gly Ala Gly Gly Ala Gly Arg Lys Glu Asp Trp Asn Glu Ile  $\frac{1}{40}$ Asp Pro Ile Lys Lys Lys Asp Leu His His Ser Asn Gly Glu Glu Lys 50 60 Ala Gln Ser Met Glu Thr Leu Pro Pro Gly Lys Val Arg Trp Pro Asp 65 70 75 80 Phe Asn Gln Glu Ala Tyr Val Gly Gly Thr Met Val Arg Ser Gly Gln
85 90 95 Asp Pro Tyr Ala Arg Asn Lys Phe Asn Gln Val Glu Ser Asp Lys Leu 100 105 110 Arg Met Asp Arg Ala Ile Pro Asp Thr Arg His Asp Gln Cys Gln Arg 115 120 125 Lys Gln Trp Arg Val Asp Leu Pro Ala Thr Ser Val Val Ile Thr Phe His Asn Glu Ala Arg Ser Ala Leu Leu Arg Thr Val Val Ser Val Leu 155 160 Lys Lys Ser Pro Pro His Leu Ile Lys Glu Ile Ile Leu Val Asp Asp 165 170 175 Tyr Ser Asn Asp Pro Glu Asp Gly Ala Leu Leu Gly Lys Ile Glu Lys Val Arg Val Leu Arg Asn Asp Arg Arg Glu Gly Leu Met Arg Ser Arg 195 200 205 Val Arg Gly Ala Asp Ala Ala Gln Ala Lys Val Leu Thr Phe Leu Asp 210 215 220 Ser His Cys Glu Cys Asn Glu His Trp Leu Glu Pro Leu Leu Glu Arg 24Ō Val Ala Glu Asp Arg Thr Arg Val Val Ser Pro Ile Ile Asp Val Ile 245 250 255 Asn Met Asp Asn Phe Gln Tyr Val Gly Ala Ser Ala Asp Leu Lys Gly 260 265 270 Gly Phe Asp Trp Asn Leu Val Phe Lys Trp Asp Tyr Met Thr Pro Glu 275 280 285

#### FIG. 13A SUBSTITUTE SHEET (RULE 26)

14/54 Gln Arg Arg Ser Arg Gln Gly Asn Pro Val Ala Pro Ile Lys Thr Pro 295 Met Ile Ala Gly Gly Leu Phe Val Met Asp Lys Phe Tyr Phe Glu Glu Leu Gly Lys Tyr Asp Met Met Met Asp Val Trp Gly Glu Asn Leu 330 Glu Ile Ser Phe Arg Val Trp Gln Cys Gly Gly Ser Leu Glu Ile Ile 345 Pro Cys Ser Arg Val Gly His Val Phe Arg Lys Gln His Pro Tyr Thr 360 Phe Pro Gly Gly Ser Gly Thr Val Phe Ala Arg Asn Thr Arg Arg Ala Ala Glu Val Trp Met Asp Glu Tyr Lys Asn Phe Tyr Tyr Ala Ala Val 395 Pro Ser Ala Arg Asn Val Pro Tyr Gly Asn Ile Gln Ser Arg Leu Glu Leu Arg Lys Leu Ser Cys Lys Pro Phe Lys Trp Tyr Leu Glu Asn 425 Val Tyr Pro Glu Leu Arg Val Pro Asp His Gln Asp Ile Ala Phe Gly Ala Leu Gln Gln Gly Thr Asn Cys Leu Asp Thr Leu Gly His Phe Ala 450 Asp Gly Val Val Gly Val Tyr Glu Cys His Asn Ala Gly Gly Asn Gln 475 Glu Trp Ala Leu Thr Lys Glu Lys Ser Val Lys His Met Asp Leu Cys 495 490 485 Leu Thr Val Val Asp Arg Ala Pro Gly Ser Leu Ile Lys Leu Gln Gly 500 505 Cys Arg Glu Asn Asp Ser Arg Gln Lys Trp Glu Gln Ile Glu Gly Asn Ser Lys Leu Arg His Val Gly Ser Asn Leu Cys Leu Asp Ser Arg Thr Ala Lys Ser Gly Gly Leu Ser Val Glu Val Cys Gly Pro Ala Leu Ser

#### FIG. 13A (CONT.)

555

560

SUBSTITUTE SHEET (RULE 26)

550

Gln Gln Trp Lys Phe Thr Leu Asn Leu Gln Gln

545

15/54

Nucleic acid	l sequence			L - I I-		<b>C</b> O
atgoggogg	c gctagaggat					60
tactacatg	t actoggggg	agatatgag	ctggccgggg	dadaddada	agagaggc	120
aggaaggag	g actggaatga	aattgaccc	attaaaaaga	aagacettea	tcacagcaat	180
ggagaagag	a aagcacaaag	catggagacc	ctactacag	ggaaagtacg	gtggccagac	240
tttaaccag	g aagettatgt	tggagggacg	atggtæget	ccgggcagga	cocttacgcc	300
ogcaacaag	t tcaaccaggt	ggagagtgat	aagcttogaa	tggacagagc	catocctgac	360
accoggcat	g accagtgtca	goggaagcag	tggagggtgg	atctgooggc	caccagogtg	420
gtgatcacg	t ttcacaatga	agocaggtog	gccctactca	ggaccgtggt	cagogtgctt	480
aagaaaagc	c ogcoccatet	cataaaagaa	atcatcttgg	tggatgacta	cagcaatgat	540
octgaggac	g gggetetett	ggggaaaatt	gagaaagtgc	gagttettag	aaatgatoga	600
cgagaaggc	c tcatgogoto	acgggttcgg	ggggccgatg	ctgcccaagc	caaggtootg	660
acettecto	g acagtcactg	ogagtgtaat	gagcactggc	tggagococt	octggaaagg	720
gtggcggag	g acaggactog	ggttgtgtca	cocatcatog	atgtcattaa	tatggacaac	780
tttcagtat	g tgggggcato	: tgctgacttg	aagggcggtt	ttgattggaa	cttggtattc	840
aagtgggat	t acatgacgcc	: tgagcagaga	. aggtææggc	aggggaaccc	agtagaact	900
ataaaaac	oc coatgattgo	tggtgggctg	tttgtgatgg	ataagtteta	ttttgaagaa	960
ctggggaag	gt acgacatgat	: gatggatgtg	tggggaggag	agaacctaga	gatctcgttc	1020
agagtatag	gc agtgtggtgg	r cagoctggag	atcatcoogt	gcagcogtgt	. gggacacgtg	1080
ttccggaag	ge ageacceta	a cacgttcccg	ggtggcægtg	gæctgtett	tgooogaaac	1140
accegoog	gg cagcagaggt	ctggatggat	. gaatacaaaa	atttctatta	. tgcagcagtg	1200
ættetge	ta gaaacgttoo	ttatggaaat	: attcagagca	. gattggagct	. taggaagaaa	1260
ctcagctg	ca agoctttcaa	a atggtacett	; gaaaatgtct	. atccagagtt	. aagggttoca	1320
gaccatca	gg atatagetti	tggggaette	r cagcagggaa	. ctaactgoot	: cgacactttg	1380
ggacactt	tg ctgatggtgl	ggttggagtt	: tatgaatgtc	: acaatgctgc	gggaaaccag	1440
gaatgggo	ct tgacgaagg	a gaagtoggto	y aagcacatgg	atttgtgcct	tactgtggtg	1500
gaccgggc	ac ogggetete	t tataaagctg	g cagggetgee	: gagaaaatga	ı cagcagacag	1560
aaatggga	ac agatogagg	g caactocaag	g ctgaggcacg	tgggcagcaa	a cetgtgeetg	1620
	ca. oggocaaga					
cagcagtg	ga agttcacgc	t caacetgeag	g cag			1713

#### FIG. 13B SUBSTITUTE SHEET (RULE 26)

16/54 Δ51 UDP-N-acetylgalactosaminyltransferase 2, GalNAcT2, nucleic acid and amino acid sequences

Amino acid sequence Lys Lys Lys Asp Leu His His Ser Asn Gly Glu Glu Lys Ala Gln Ser 1 10 15 Met Glu Thr Leu Pro Pro Gly Lys Val Arg Trp Pro Asp Phe Asn Gln 20 25 30 Glu Ala Tyr Val Gly Gly Thr Met Val Arg Ser Gly Gln Asp Pro Tyr 35 40 45Ala Arg Asn Lys Phe Asn Gln Val Glu Ser Asp Lys Leu Arg Met Asp 50 60 Arg Ala Ile Pro Asp Thr Arg His Asp Gln Cys Gln Arg Lys Gln Trp 65 75 80 Arg Val Asp Leu Pro Ala Thr Ser Val Val Ile Thr Phe His Asn Glu 85 90 95 Ala Arg Ser Ala Leu Leu Arg Thr Val Val Ser Val Leu Lys Lys Ser 100 105 110 Pro Pro His Leu Ile Lys Glu Ile Ile Leu Val Asp Asp Tyr Ser Asn 115 120 Asp Pro Glu Asp Gly Ala Leu Leu Gly Lys Ile Glu Lys Val Arg Val 130 135 Leu Arg Asn Asp Arg Glu Gly Leu Met Arg Ser Arg Val Arg Gly 145 150 150 Ala Asp Ala Ala Gln Ala Lys Val Leu Thr Phe Leu Asp Ser His Cys 165 170 Glu Cys Asn Glu His Trp Leu Glu Pro Leu Glu Arg Val Ala Glu 180 185 190 Asp Arg Thr Arg Val Val Ser Pro Ile Ile Asp Val Ile Asn Met Asp 195 200 205 Asn Phe Gln Tyr Val Gly Ala Ser Ala Asp Leu Lys Gly Gly Phe Asp 210 215 220 Trp Asn Leu Val Phe Lys Trp Asp Tyr Met Thr Pro Glu Gln Arg Arg 225 230 235 Ser Arg Gln Gly Asn Pro Val Ala Pro Ile Lys Thr Pro Met Ile Ala 245 255 Gly Gly Leu Phe Val Met Asp Lys Phe Tyr Phe Glu Glu Leu Gly Lys 260 270 Tyr Asp Met Met Met Asp Val Trp Gly Gly Glu Asn Leu Glu Ile Ser 275 280 285 Phe Arg Val Trp Gln Cys Gly Gly Ser Leu Glu Ile Ile Pro Cys Ser

#### FIG. 14A SUBSTITUTE SHEET (RULE 26)

#### 17/54

	290					295					300				
Arg 305	Val	Gly	His	Val	Phe 310	Arg	Lys	Gln	His	Pro 315	Tyr	Thr	Phe	Pro	Gly 320
Gly	Ser	Gly	Thr	Val 325	Phe	Ala	Arg	Asn	Thr 330	Arg	Arg	Ala	Ala	Glu 335	Val
Trp	Met	Asp	Glu 340	Tyr	Lys	Asn	Phe	Tyr 345	Tyr	Ala	Ala	Val	Pro 350	Ser	Ala
Arg	Asn	Val 355	Pro	Tyr	Gly	Asn	Ile 360	Gln	Ser	Arg	Leu	Glu 365	Leu	Arg	Lys
Lys	Leu 370	Ser	Cys	Lys	Pro	Phe 375	Lys	Trp	Tyr	Leu	Glu 380	Asn	Val	Tyr	Pro
Glu 385	Leu	Arg	Val	Pro	Asp 390	His	Gln	Asp	Ile	Ala 395	Phe	Gly	Ala	Leu	Gln 400
Gln	Gly	Thr	Asn	Cys 405	Leu	Asp	Thr	Leu	Gly 410	His	Phe	Ala	Asp	Gly 415	Val
Val	Gly	Val	Tyr 420	Glu	Cys	His	Asn	Ala 425	Gly	Gly	Asn	Gln	Glu 430	Trp	Ala
Leu	Thr	Lys 435	Glu	Lys	Ser	Val	Lys 440	His	Met	Asp	Leu	Cys 445	Leu	Thr	Val
Val	Asp 450	Arg	Ala	Pro	Gly	Ser 455	Leu	Ile	Lys	Leu	Gln 460	Gly	Cys	Arg	Glu
Asn 465	Asp	Ser	Arg	Gln	Lys 470	Trp	Glu	Gln	Ile	Glu 475	Gly	Asn	Ser	Lys	Leu 480
Arg	His	Val	Gly	Ser 485	Asn	Leu	Cys	Leu	Asp 490	Ser	Arg	Thr	Ala	Lys 495	Ser
Gly	Gly	Leu ·	Ser 500	Val	Glu	Val	Cys	Gly 505	Pro	Ala	Leu	Ser	Gln 510	Gln	Trp
Lys	Phe	Thr 515		Asn	Leu	Gln	Gln 520								

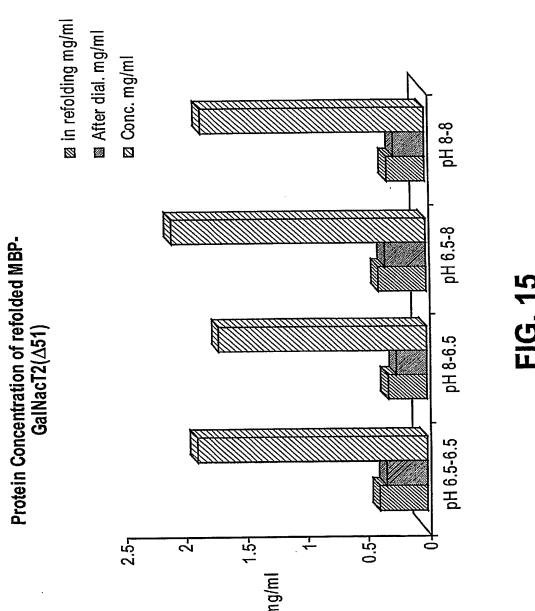
#### **FIG. 14A (CONT.)**

#### **SUBSTITUTE SHEET (RULE 26)**

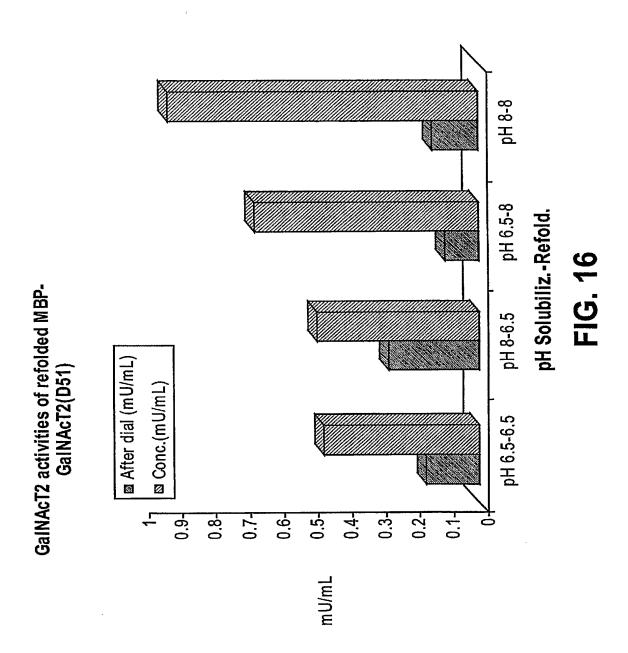
Nucleic acid s	sequence		18/54			
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cctccaggga	aagtacggtg	gocagacttt	aaccaggaag	cttatgttgg	agggacgatg	120
gtægetæg	ggcaggaccc	ttagoogc	aacaagttca	accaggtgga	gagtgataag	180
cttogaatgg	acagagccat	catgacacc	oggcatgacc	agtgtcagog	gaagcagtgg	240
agggtggatc	tgaagaac	cagogtggtg	atcacgtttc	acaatgaagc	æggtægæ	300
ctactcagga	cogtggtczg	ogtgettaag	aaaagcccgc	cocatctcat	aaaagaaatc	360
atcttggtgg	atgactacag	caatgatoct	gaggacgggg	ctctcttggg	gaaaattgag	420
aaagtgcgag	ttcttagaaa	tgatogaoga	gaaggootoa	tgagatææg	ggttagggg	480
gccgatgctg	cocaagocaa	ggtætgaæ	ttoctggaca	gtæctgæga	gtgtaatgag	540
cactggctgg	agoooctoct	ggaaagggtg	gaggaggaca	ggactogggt	tgtgtcaccc	600
atcatcgatg	tcattaatat	ggacaacttt	cagtatgtgg	gggcatctgc	tgacttgaag	660
ggaggttttg	attggaactt	ggtattcaag	tgggattaca	tgacgcctga	gcagagaagg	720
taaggazgg	ggaacocagt	ogococtata	aaaaccccca	tgattgctgg	tgggctgttt	780
gtgatggata	agttetattt	tgeagaactg	gggaagtacg	acatgatgat	ggatgtgtgg	840
ggaggagaga	acctagagat	ctagttage	gtgtggægt	gtggtggæg	octggagatc	900
atcocgtgca	. googtgtggg	acacgtgttc	oggaagcagc	accetacac	gttææggt	960
ggcagtggca	. ctgtctttgc	cogaaacacc	: agaagggaag	cagaggtctg	gatggatgaa	1020
tacaaaaatt	tctattatgc	agcagtgcct	. tctgctagaa	acgttcctta	. tggaaatatt	1080
cagagcagat	. tggagettag	gaagaaactc	: agetgeaage	ctttcaaatg	gtaccttgaa	1140
aatgtotato	: cagagttaag	ggttæagac	: catcaggata	tagettttgg	genetten	1200
cagggaacta	actgoctoga	. cactttggga	. cactttgctg	atggtgtggt	. tggagtttat	1260
gaatgtcaca	atgctgggg	aaaocaggaa	ı tgggaettga	. cgaaggagaa	, gtaggtgaag	1320
cacatggatt	tgtgccttac	: tgtggtggad	c agggczaagg	getetettat	: aaagctgcag	1380
ggetgeogæ	g aaaatgacag	r cagacagaaa	ı tgggaacaga	i togagggcaa	ı ctocaagetg	1440
aggcacgtg	g gcagcaacct	: gtgaatggad	agtagaagg	ocsagagogo	g gggcctaagc	1500
gtggaggtgt	z gtggcccggc	cottogoaq	g cagtggaagt	tcagetca	a octgoagoag	1560

FIG. 14B

**SUBSTITUTE SHEET (RULE 26)** 

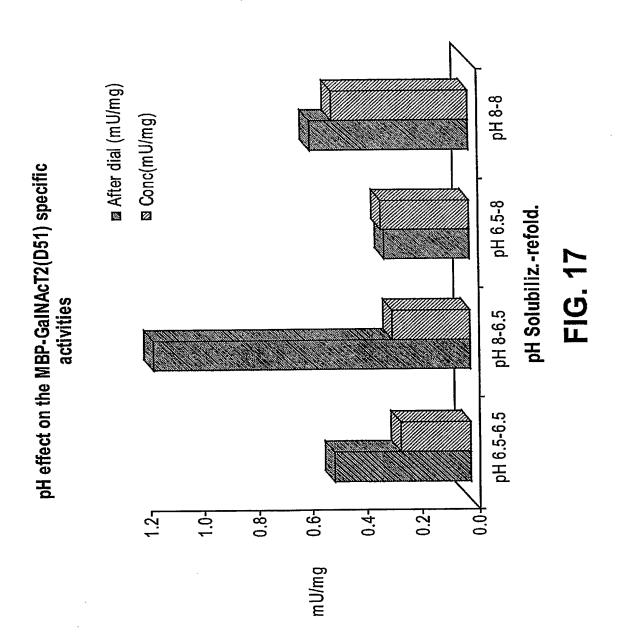


20/54

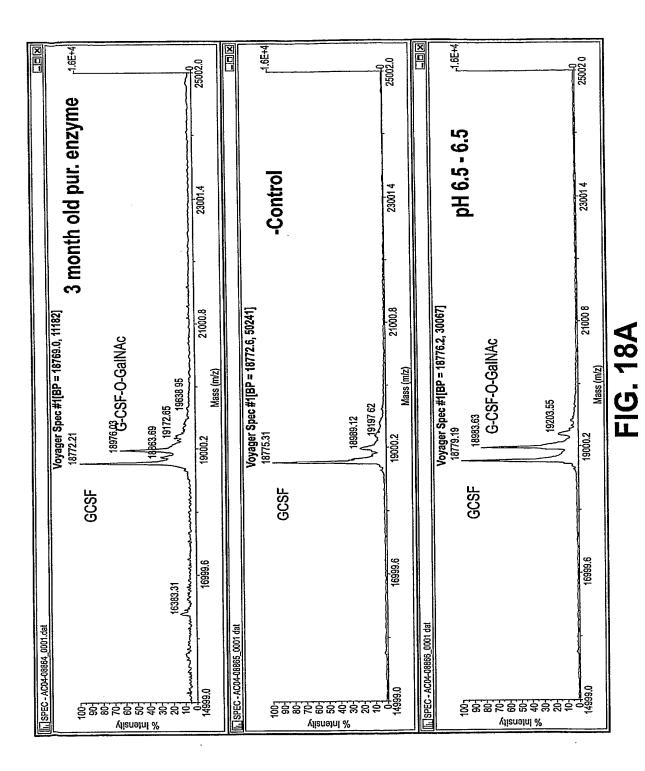


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**SUBSTITUTE SHEET (RULE 26)** 

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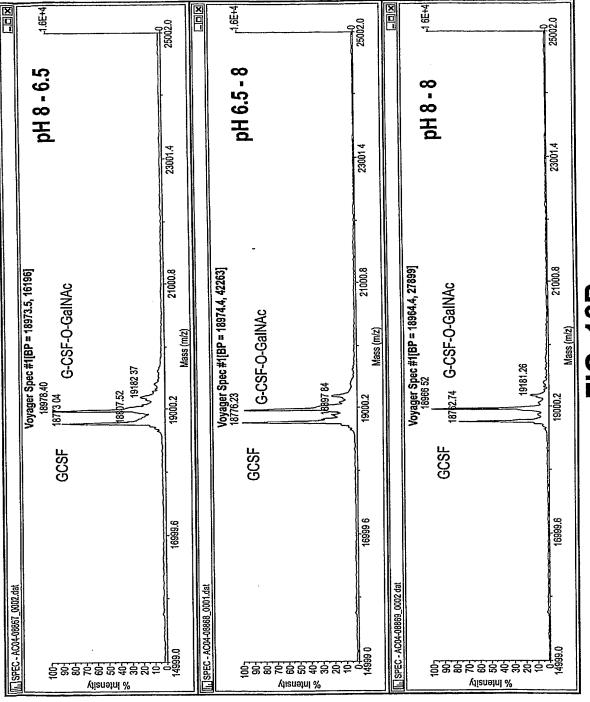
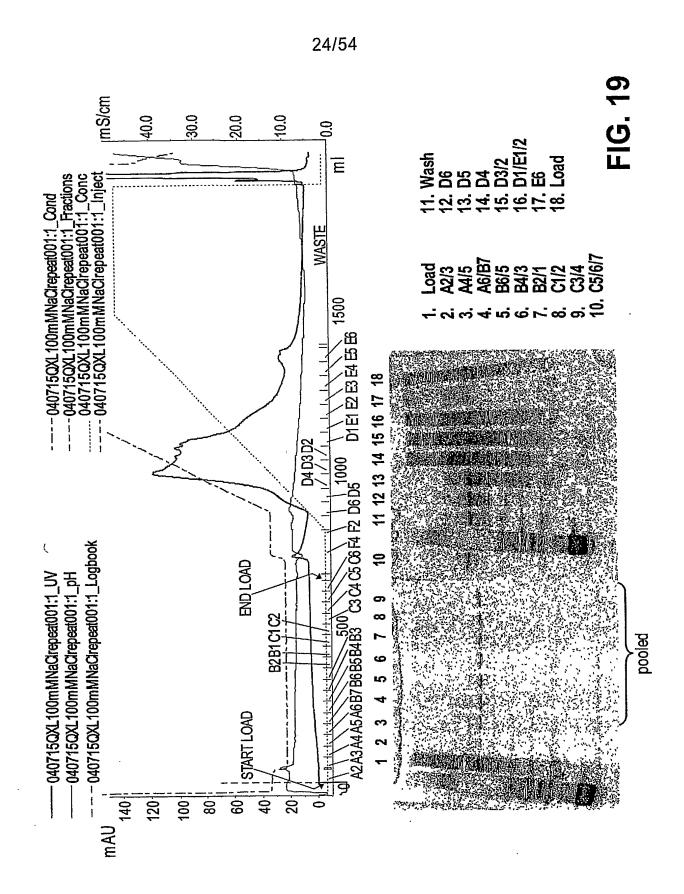


FIG. 18B

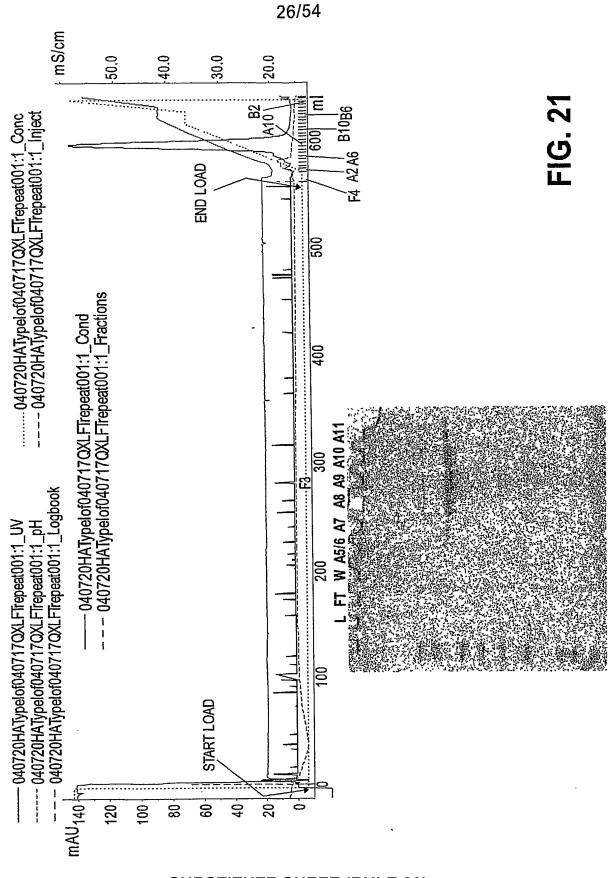


**SUBSTITUTE SHEET (RULE 26)** 

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	Volume (mL)	Activity (U/L)	A280
Load	890	1.5	0.110
FT <sub>A4-C4</sub>	670	9.2	NA
FT <sub>C5-C7</sub>	120	1.0	0
Wash	138	3.6	0.100
D6	45	4.5	0
D5	45	2.4	0.026
D4	45	2.0	0.108
D3/2	90	1.1	0.179
E6		0.0	0.017

FIG. 20



**SUBSTITUTE SHEET (RULE 26)** 

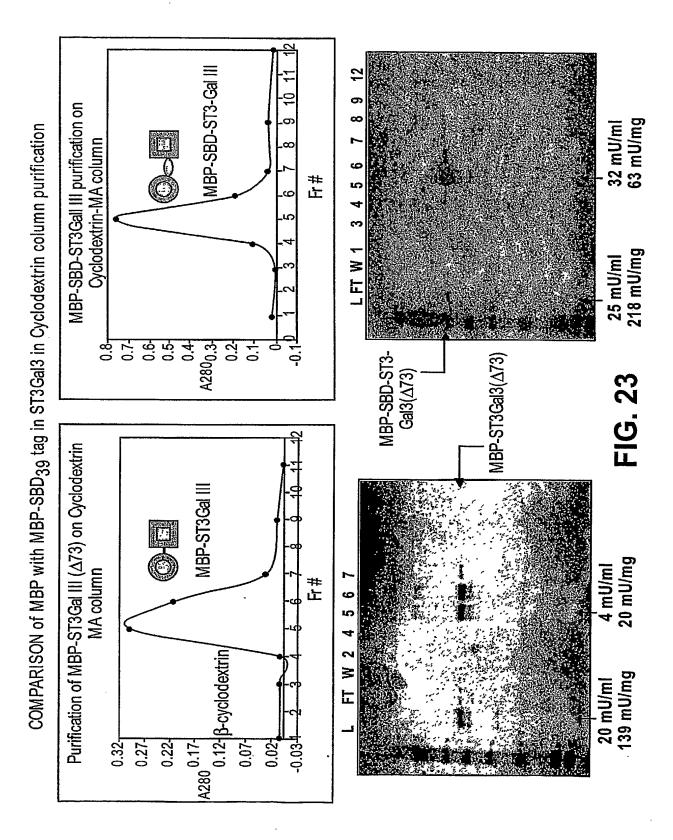
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Pooled A4-C4 and adjusted pH to 7.0 using 1mM HCl 670mL load pH 7.0 16mS/cm

	Volume (mL)	Activity (U/L) pre-	Activity (U/L) post- dialysis	A280	A280/ 1.51 (mg/mL)	Activity (U)	Mass (mg)	Specific Activity (U/mg)
Load	029	9.2	NA	AN AN				
FT	670	0.0	AN	0.122				
Wash	6	2.9	NA	-0.013				
A5/6	9	1.1	NA	-0.005				
A7	3	0.1						
A8	3	1.3	,					
A9	3	4.6	19.3 (13ml)	0.180	0.119	0.25	1.55	0.16
A10	3	2.4	(101115)					
A11	3	0.4						

FIG. 22

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#### **SUBSTITUTE SHEET (RULE 26)**

GNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPOIDSHDFVLRMNKAPTEGFEADVGSKTTHHFVYPE KELKAKGKSALMFNLOEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP PCTCTRCIEEORVSAWFDERFNRSMQPLLTAKNAHLEEDTYKWWLRLQREKQPNNLNDTIRELFQVVP WYAVRTAVINAASGROTVDEALKDAOTNSSSINININININININLGIEGRISEFGSELSENFKKLMKYPYR <u>GRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDFESNVTTILASIN</u> NKELAKEFLENYLITDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF SFREL A OEVSMIL V PFKTTDLEWVISATTTGRISHT YV PVPAKIK V KKEKIL I YHPAFIKY V FDRWL QGH GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD

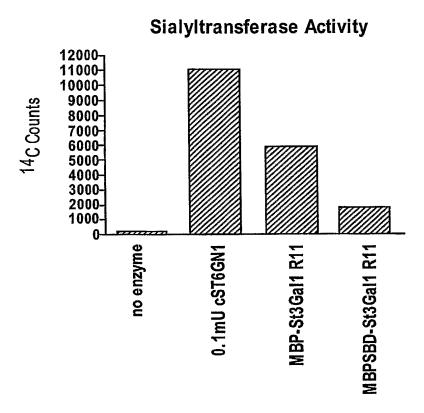
MBP-pST3Gal1 fusion protein

## IG. 24A

MBPSBD-pST3Gal1 fusion protein

KELKAKGKSALMFNLOEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP FKKLMKYPYRPCTCTRCIEEORVSAWFDERFNRSMOPLLTAKNAHLEEDTYKWWLRLOREKOPNNLN DTIRELFQVVPGNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPQIDSHDFVLRMNKAPTEGFEADVG *YVFDRWLQGHGRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDF* NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAOKGEIMPNIPOMSAF KYTSSDPLWYVTVTLPAGESFEYKFIRIESDDSVEWESDPNREYTVPQACGTŠTATVTDTWRGSELSEN MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPOVAATGDGPDIIFWAHDRF SKTTHHFVYPESFRELAOEVSMILVPFKTTDLEWVISATTTGRISHTYVPVPAKIKVKKEKILIYHPAFIK GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLJAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGSIVATGGTTTTTATPTG SGSVTSTSKTTATASKTŠTSTSSTSCTTPTAVAVTFDLTATTTYGENIYLVGSISQLGDWETSDGIALSAD ESNVTTILASINKIRIFKGR

FIG. 24B



Test pST3Gal1 for activity after Hampton refold

FIG. 25

KELKAKGKSALMFNLQEPYFTWPLJAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLJKNKH NCSASVKIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSLVQKVVTRFP HRPQEAFRNALDLDRYLLLHPDFLRYMKNRFLRSKTLDTAXWRIYRPTTGALLLLTALHLCDKV MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP STRKTPMATGAVPAKKKVVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEEYSLDMSSLQT SFYGFTAFSLTQSILILGRRGFQHVPLGKDVRYLHFLEGTRNYEWLEAMFLNQTLAKTHLSWFR NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF PVRQQQLLLASL.PTGYSKCITCAVVGNGGILNDSRVGREIDSHDYVFRLSGAVIKGYEQDVGTRT WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGSSEHLDKVPRTPGAL SAYGFITEGHERFSDHYYDTSWKRLIFYINHDFRLERMVWKRLHDEGIIWLYQRPQSDKAKN

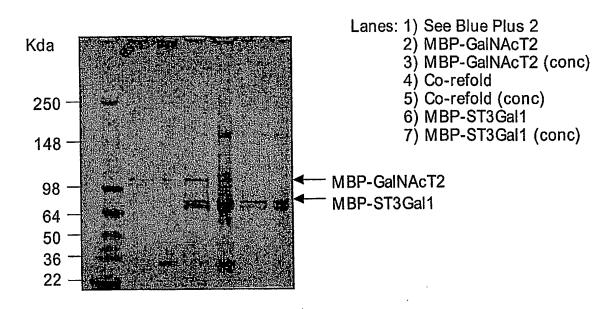
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPOVAATGDGPDIIFWAHDRF

GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD

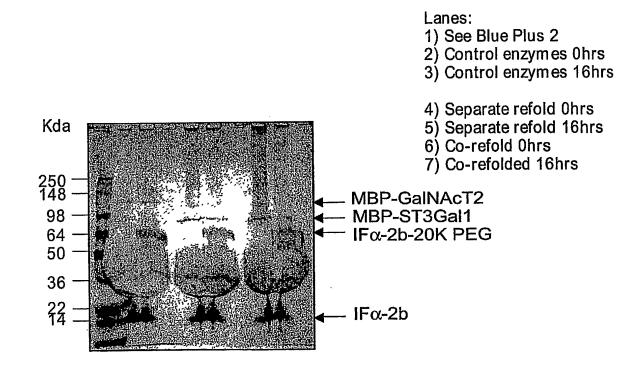
## FIG. 26A

SPITIQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSVKIKASKSLWLQKLFLPNLTLFLDS GILNNSHMGQEIDSHDYVFRLSGALIKGYEQDVGTRTSFYGFTAFSLTQSLLILGNRGFKNVPLGK **DVRYLHFLEGTRDYEWLEALLMNOTVMSKNLFWFRHRPQEAFREALHMDRYLLLHPDFLRYM** KNRFLRSKTLDGAHWRIYRPTTGALLLLTALQLCDQVSAYGFTTEGHERFSDHYYDTSWKRLIFY KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH NIKERSL QSL AKPKSQAPTRARRITIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP **VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNGGQTRKLTA** RHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVTRFPPVPQQQLLLASLPAGSLRCITCAVVGNG NKELAKEFLENYL LTDEGLEAVNKDKPLGAVALKSYEEFLAKDPRIAATMENAQKGEIMPNIPQMSAF WYAVRIAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGSKEPQTKPSRHQRTE SRIVSEKHQGKAATTAKTLIPKSQHRMLAPTGAVSTRIRQKGVTTAVIPPKEKKPQATPPPAPFQ MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD NHDFKLEREVWKRLHDEGIIRLYQRPGPGTAKAKN

## FIG. 26B

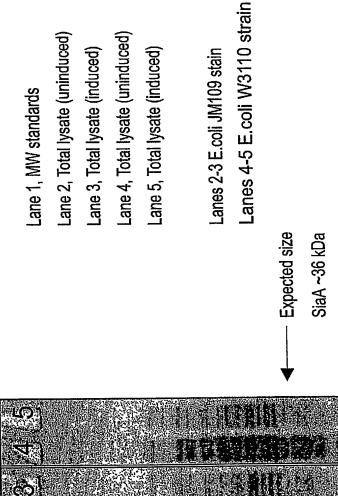


**FIG. 27A** 



**FIG. 27B** 

## Induced SiaA expression in E.coli



22 kDa 🕒

16 KDa —

6 kDa

There is no obvious inducible band at the expected

Mass of 36 kDa for the native SiaA protein.

FIG. 28

50 kDa —

64 kDa

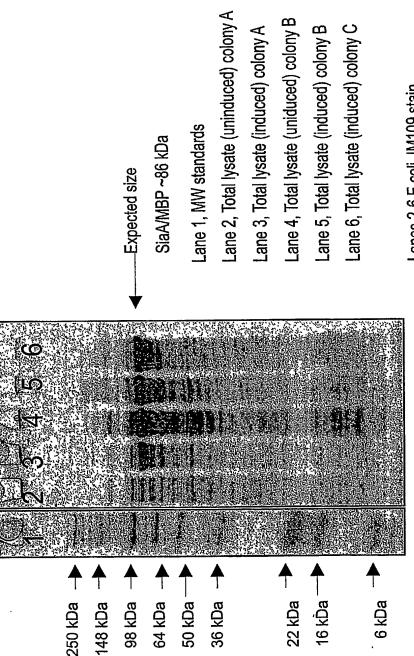
98 kDa

250 kDa

148 kDa

36 kDa

# Induced SiaA/MBP expression in E.coli



Lanes 2-6 E.coli JM109 stain

Compare to figure X where SiaA production is not obvious. The presence of High level production of SiaA/MBP even in absence of IPTG induction. the fusion partner (MBP) drives high levels of expression.

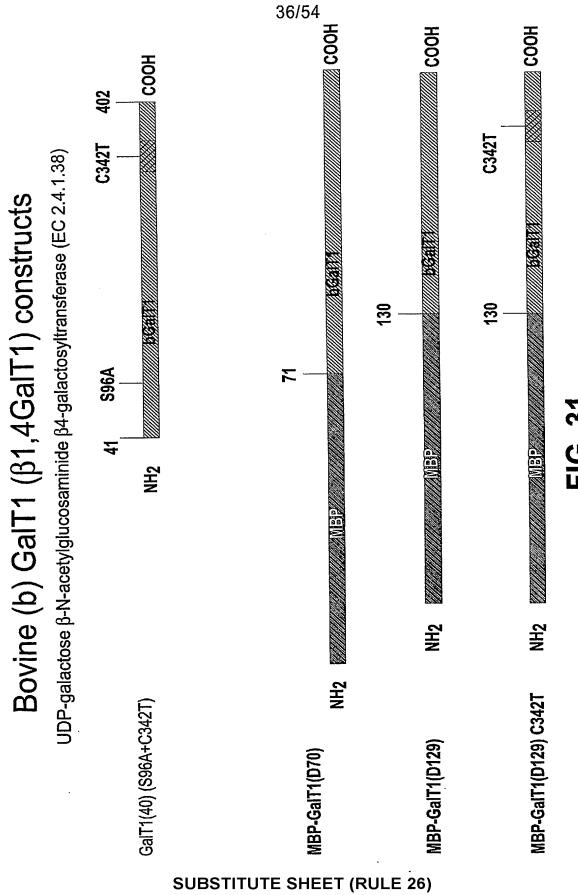
FIG. 29

ggvsalskqq kknepnpgrf gpgpgsnlts ytpmdcisph llnvgfkeal pqlvgvhppl agesmfnrak kcrmirhsrd alhlgvtlvy ylagrdlrrl nldayshpgp gfslpyvqyf qnpkvklggr sskprsraps ipvdlklieg svsrpnavig ytkitvdigt ldygiyving rhisvamdkf hntyrcfsqp mvlevqrypl lvgpmliefn ylhpmvqrqq iynrlafrgm racrllvavc gvapppplqn ywgwggeddd rqehlkywly sdvdlipmnd lsdglnslty saampgaslq gqpsgelrlr ltacpeespl flsingfpnn driahtketm kvaiiilfrn kdydyncfvf apvpstttrs mkfrepllgg qgsshgaaai

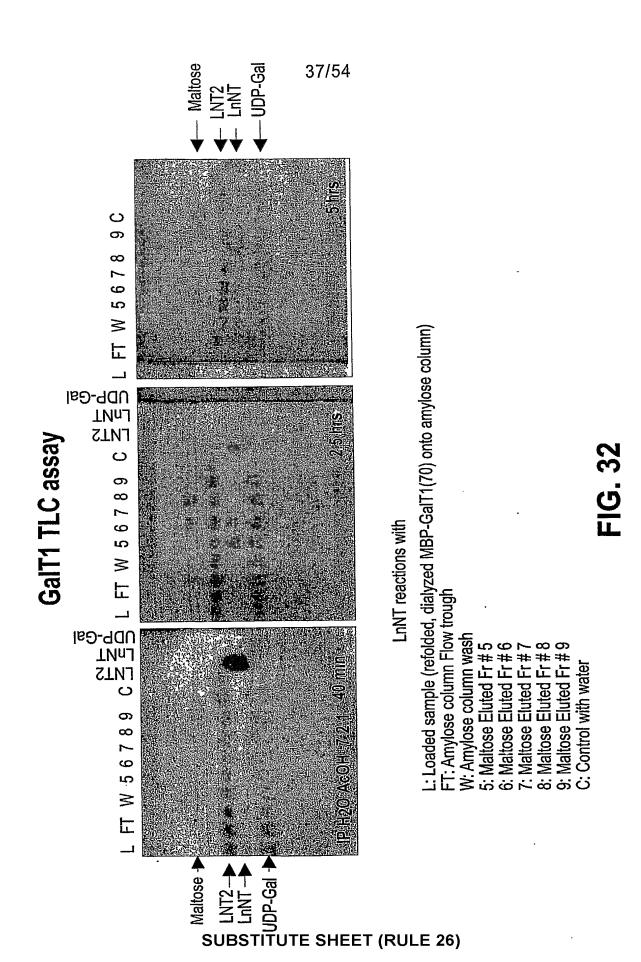
FIG. 3(

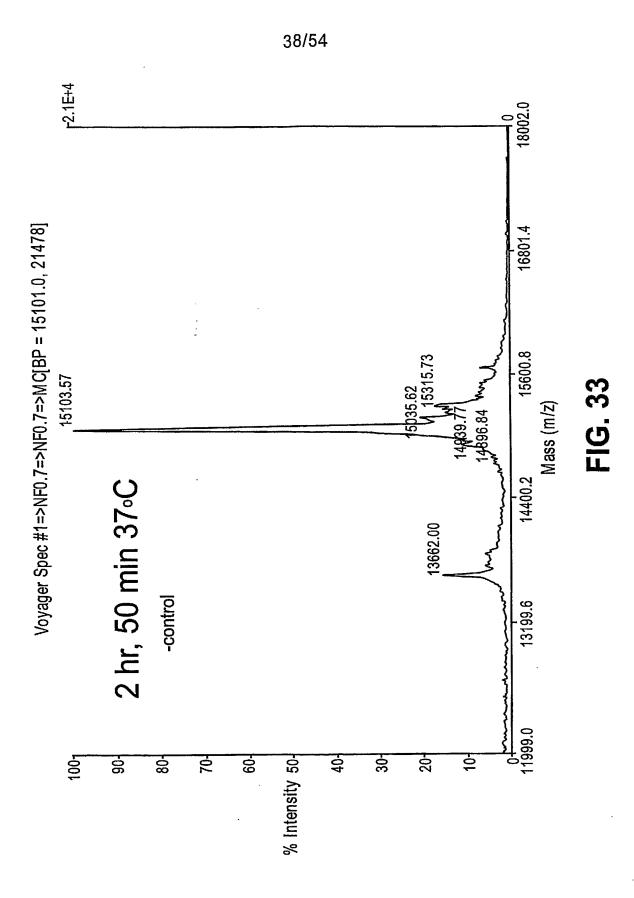
181

241 301 361

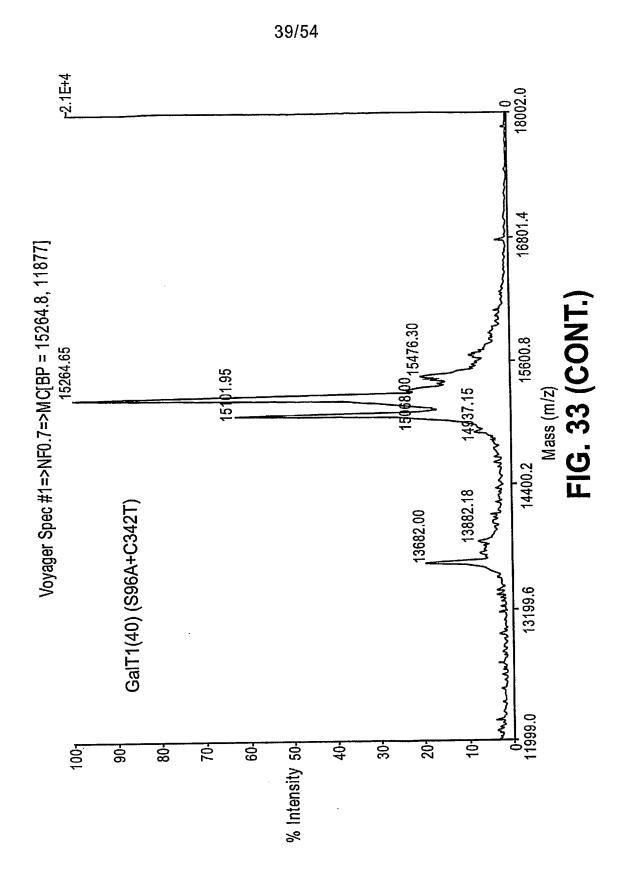


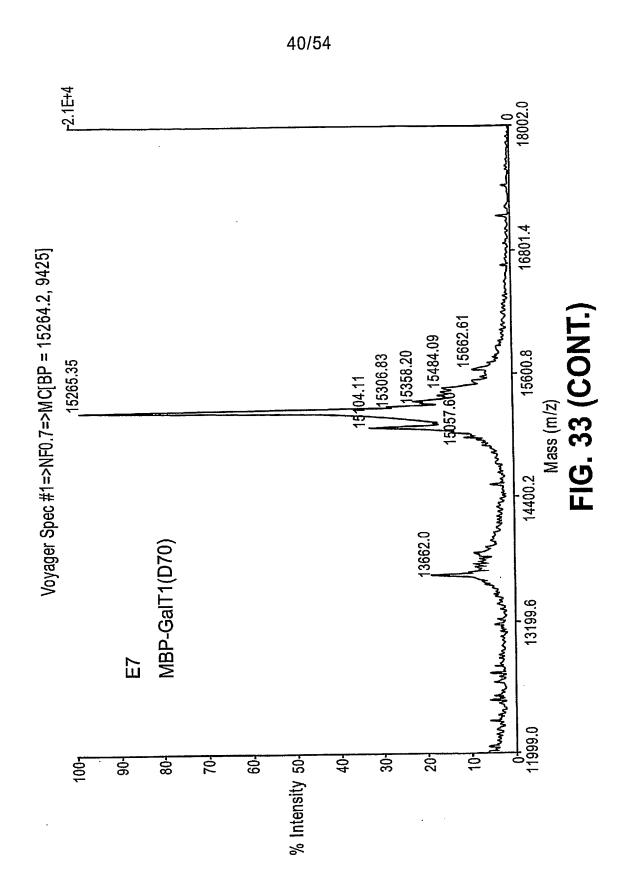
FG. 3

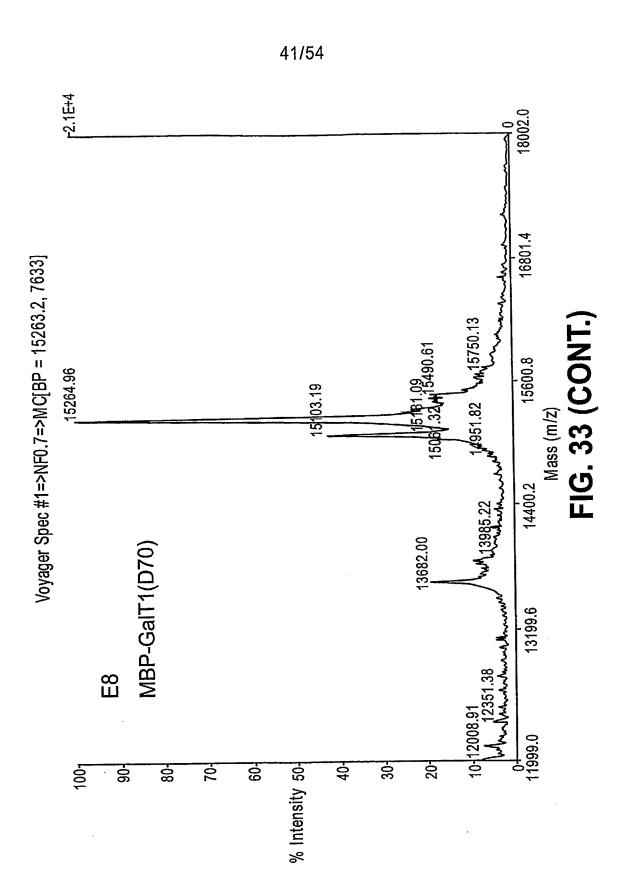




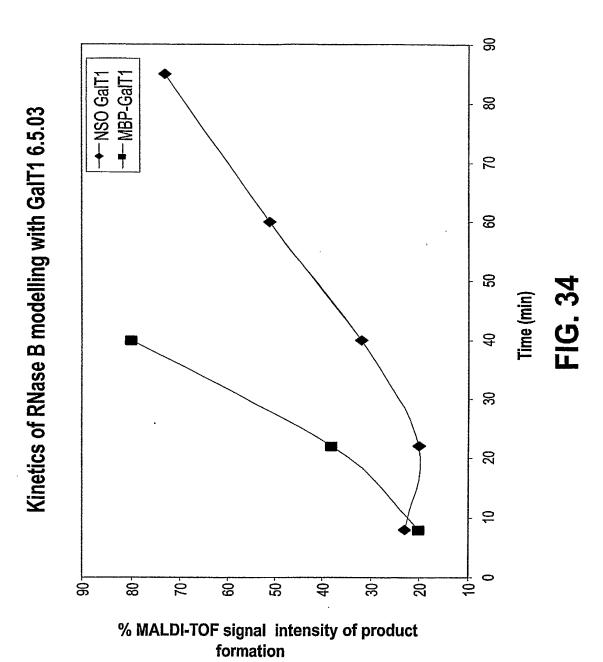
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**SUBSTITUTE SHEET (RULE 26)** 

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**GnT1** constructs

MBP-GnT1(A35)

MBP-GnT1(∆103)

mlkkqsaglv lwgailfvaw nallllffwt rpapgrppsv saldgdpasl trevirlagd

121 cldkllhyrp saelfpiivs qdcgheetaq aiasygsavt hirqpdlssi avppdhrkfq aevelerqrg llqqigdals sqrgrvptaa ppaqprvpvt papavipilv iacdrstvrr

181 gyykiarhyr walgqvfrqf rfpaavvved dlevapdffe yfratypllk adpslwcvsa 241 wndngkeqmv dasrpellyr tdffpglgwl llaelwaele pkwpkafwdd wmrrpeqrgg 301 racirpeisr tmtfgrkgvs hgqffdqhlk fiklnqqfvh ftqldlsylq reaydrdfla

361 rvygapqlqv ekvrtndrke lgevrvqytg rdsfkafaka lgvmddlksg vpragyrgiv

421 tfqfrgrrvh lappptwegy dpswn



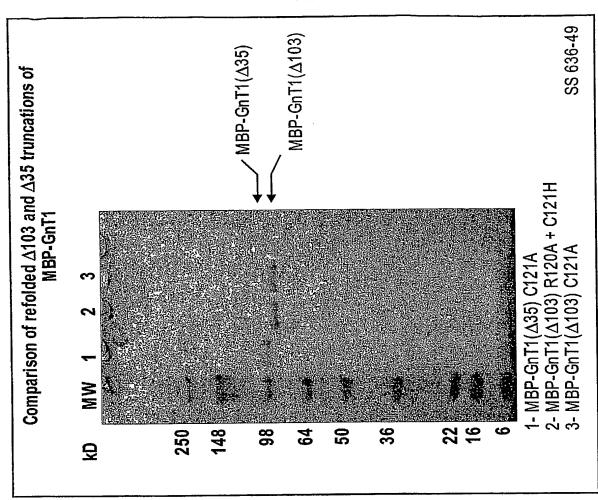
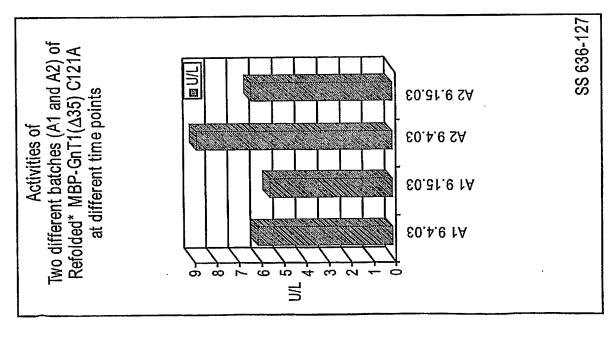


FIG. 36



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WO 2005/089102

gfgadskgnw fkklmkypyr kqpnnlndti isatttgtis ykwwlrlgre pqidshdfvl kqmvielsen 1hicdevdly stgilsvifs htvvttawfp aknahleedt sgnlkesyyg pfkttdlewv lasinkirif lagevsmilv rwlqghgryp scrrcavvgn gdfesnvtti fnrsmqpllt fltsfflnys kvkkekiliy hpafikyvfd gafrktgvhd vdpllekrlv hfvypesfre qrvsawfder klltllvlfi eadvgsktth hhywennpsa relfqvvpgn pctctrciee mapmrkkstl 61 121 181 241 301

FIG. 3

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Human ST6GalNAcI
MRSCLWRCRHLSQGVQWSLLLAVLVFFLFALPSFIKEPQTKPSRHQRTENIKERSLQS
LAKPKSQAPTRARRTTIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK
VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNG
GQTRKLTASRTVSEKHQGKAATTAKTLIPKSQHRMLAPTGAVSTRTRQKGVTTAVIP
PKEKKPQATPPPAPFQSPTTQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSV
KIKASKSLWLQKLFLPNLTLFLDSRHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVT
RFPPVPQQQLLLASLPAGSLRCITCAVVGNGGILNNSHMGQEIDSHDYVFRLSGALIK
GYEQDVGTRTSFYGFTAFSLTQSLLILGNRGFKNVPLGKDVRYLHFLEGTRDYEWLE
ALLMNQTVMSKNLFWFRHRPQEAFREALHMDRYLLLHPDFLRYMKNRFLRSKTLD
GAHWRIYRPTTGALLLLTALQLCDQVSAYGFITEGHERFSDHYYDTSWKRLIFYINH
DFKLEREVWKRLHDEGIIRLYQRPGPGTAKAKN

#### **FIG. 38A**

Chicken ST6GalNAcI
MGFLIRRLPKDSRIFRWLLILTVFSFIITSFSALFGMEKSIFRQLKIYQSIAHMLQVDTQ
DQQGSNYSANGRISKVGLERDIAWLELNTAVSTPSGEGKEEQKKTVKPVAKVEEAK
EKVTVKPFPEVMGITNTTASTASVVERTKEKTTARPVPGVGEADGKRTTIALPSMKE
DKEKATVKPSFGMKVAHANSTSKDKPKAEEPPASVKAIRPVTQAATVTEKKKLRAA
DFKTEPQWDFDDEYILDSSSPVSTCSESVRAKAAKSDWLRDLFLPNITLFIDKSYFNV
SEWDRLEHFAPPYGFMELNYSLVEEVMSRLPPNPHQQLLLANSSSNVSTCISCAVVG
NGGILNNSGMGQEIDSHDYVFRVSGAVIKGYEKDVGTKTSFYGFTAYSLVSSLQNLG
HKGFKKIPQGKHIRYIHFLEAVRDYEWLKALLLDKDIRKGFLNYYGRRPRERFDEDF
TMNKYLVAHPDFLRYLKNRFLKSKNLQKPYWRLYRPTTGALLLLTALHLCDRVSAY
GYITEGHQKYSDHYYDKEWKRLVFYVNHDFNLEKQVWKRLHDENIMKLYQRS

#### **FIG. 38B**

Mouse ST6GalNAcI protein beginning at residue 32 of the native mouse protein DPRAKDSRCQFIWKNDASAQENQQKAEPQVPIMTLSPRVHNKESTSVSSKDLKKQER EAVQGEQAEGKEKRKLETIRPAPENPQSKAEPAAKTPVSEHLDKLPRTPGALSTRKTP MATGAVPAKKKVVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEEYSLDMSSL QTNCSASVKIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSL VQKVVTRFPPVRQQQLLLASLPTGYSKCITCAVVGNGGILNDSRVGREIDSHDYVFR LSGAVIKGYEQDVGTRTSFYGFTAFSLTQSILILGRRGFQHVPLGKDVRYLHFLEGTR NYEWLEAMFLNQTLAKTHLSWFRHRPQEAFRNALDLDRYLLLHPDFLRYMKNRFL RSKTLDTAHWRIYRPTTGALLLLTALHLCDKVSAYGFITEGHQRFSDHYYDTSWKRL IFYINHDFRLERMVWKRLHDEGIIWLYQRPQSDKAKN

#### **FIG. 38C**

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Hum (h)ST6GalNAcl truncations	N600 S65 aa N600	T73 528 aa	E110	M134 467 aa	T171	A233	G273 327 aa 2	
	Λ35	Δ72	Δ109	Δ133	Δ170	Λ232	Δ272	

**上**G. 公

# MBP-hST6GalNAcl constructs

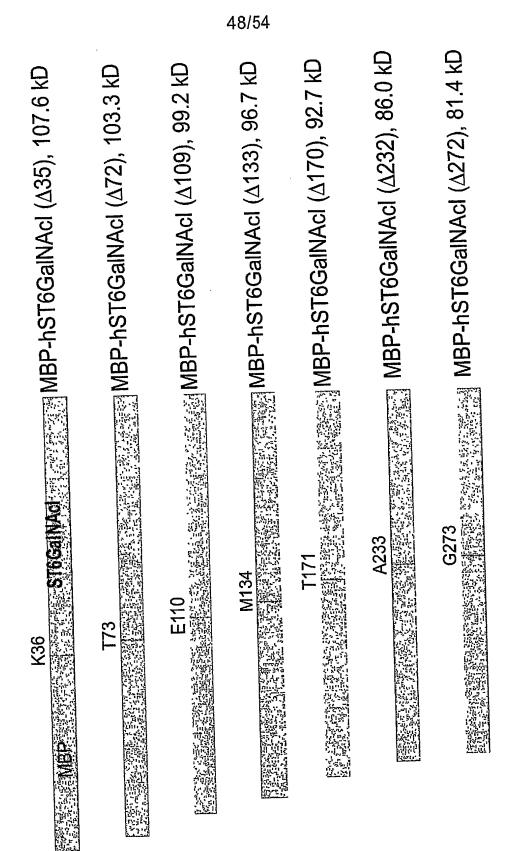


FIG. 40

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MASKSWLNFLTFLCGSAIGFLLCSQLFSILLGEKVDTQPNVLHNDPHARHSDDNGQN HLEGQMNFNADSSQHKDENTDIAENLYQKVRILCWVMTGPQNLEKKAKHVKATW AQRCNKVLFMSSEENKDFPAVGLKTKEGRDQLYWKTIKAFQYVHEHYLEDADWFL KADDDTYVILDNLRWLLSKYDPEEPIYFGRRFKPYVKQGYMSGGAGYVLSKEALKR FVDAFKTDKCTHSSSIEDLALGRCMEIMNVEAGDSRDTIGKETFHPFVPEHHLIKGYL PRTFWYWNYNYYPPVEGPGCCSDLAVSFHYVDSTTMYELEYLVYHLRPYGYLYRY QPTLPERILKEISQANKNEDTKVKLGNP

**FIG. 41** 

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		ر ر	20	100	150 150	200	250 250	300	350 350
Mode	Normal Normal	30 40	DWMEMSGPEQ DVGGHEHVHE NSTLAERLYS DWMEMSGPEQ DVGGHEHVHE NSTLAERLYS	KRC NKLIFMSSAK DD KRC NKLIFMSSAK DD	DWE ILKADDDTYTI VE DWE ILKADDDTYTI VE	180   YMSGGAGYVL   SREAVRREVV   EA   YMSGGAGYVL   SREAVRREVV   EA	230 240  LAGDSRDSNG RGRFFPFVPE HH  LAGDSRDSNG RGRFFPFVPE HH	280 DNAISFHYVS PNC	330 340 EQATESTSDG VSKRSPAETKT Q* EQATESTSDG VSKRSDETKT Q*
	342 N 342 N		GDVNDAHHSH	70 NPSNHQKKAR NPSNHQKKAR	120 KTKEAYKYIY KTKEAYKYIY	170 CKEKPYVKOG CKFKPYVKOG	220 IGKCLONVNV	270 KTDEGLDCCS KTDEGLDCCS	320 AVGELMPEIK AVGELMPEIK
Range	<u> </u>	10	国国	60 EVRVI,CWIMT EVRVI,CWIMT	110 VGEGRNNIMG VGEGRNNIMG	160 YSPETPVYFG CR		ـــــــا سبــــن	
Size	342	747	<b>근</b> 근	51	101	151	201	251 251 251	301
Segmence	SCIGALTI.AMI	SPTS122.AML	SC1GALT1.AMI SPTS122.AMI	SCIGALTI.AMI	SCIGALTI.AMI	SCIGALTI.AMI	SCIGALTI.AMI	SCIGALTI.AMI	SCIGALTI.AMI SPTS122.AMI

FIG. 42

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Yersinia MBP	)			
MKTEEGKLVI	WINGDKGYNG	LAEVGKKFEK	DTGIKVTIEH	PDKLEEKFPQ
VAATGDGPDI	IFWAHDREGG	YAOSGLLAEL	TPSKAFQEKL	FPFTWDAVRF
NGKLIGYPVA	VEALSLIYNK	DLVKEAPKTW	EEIPALDKTL	RANGKSAIMW
NLQEPYFTWP	VIAADGGYAF	KFENGVYDAK	NVGVNNAGAQ	AGLQFIVDLV
KNKHINADTD	YSIAEAAFNK	GETAMTINGP	WAWSNIDKSK	INYGVTLLPT
FHGQPSKPFV			LENYLITDQG	LAEVNKDKPL
CAVALKSFOE	OLAKOPRIAA	TMDNATNGEI	MPNIPQMAAF	WYATRSAVLN
ATTGROTVEA			_	

#### **FIG. 43A**

E. coli MBP				
MKIEEGKLVI	WINGDKGYNG	LAEVGKKFEK	DTGIKVTVEH	PDKLEEKFPQ
VAATGDGPDI	IFWAHDRFGG	YAQSGLLAEI	TPDKAFQDKL	YPFTWDAVRY
NGKTTAYPTA	VEALSLIYNK	DLLPNPPKTW	EEIPALDKEL	KAKGKSALMF
NLOEPYETWP	LIAADGGYAF	KYENGKYDIK	DVGVDNAGAK	AGLTFLVDLI
			WAWSNIDTSK	VNYGVTVLPT
		SPNKELAKEF	LENYLLTDEG	LEAVNKDKPL
		TMENAQKGEI		
AASGRQTVDE				

#### FIG. 43B

Pyrococcus furiosus MBP MKIEEGKVVI WHAMQPNELE VFQSLAEEYM ALSPEVEIVF EQKPNLEDAN						
	MKIEEGKVVI	WHAMOPNELE	VFQSLAEEYM	ALSPEVEIVE	EQKPNLEDAL	
	KAATPTGOGP	DIFIWAHDWI	GKFAEAGLLE	PIDEYVTEDL	LNEFAPMAQD	
	AMOYKGHYYA	LPFAAETVAI	IYNKEMVSEP	PKTFDEMKAI	MEKYYDPANE	
	KYGTAWPTNA	YFISATAOAF	GGYYFDDKTE	QPGLDKPETI	EGFKFFFTEI	
	WPYMAPTCDY	NTOOSTFLEG	RAPMMVNGPW	SINDVKKAGI	NFGVVPLPPI	
	TKDCKEAMBB	PYGGVKLTYE	AAGIKNKDAA	WKFAKWLTTS	EESIKTLALE	
	TCYTPUTTKV	TUDDETKNDD	VIYGFGQAVQ	HAYLMPKSPK	MSAVWGGVDG	
	AIMETLODPO	NADTEGILKK	YQQEILNNMQ	G		

FIG. 43C
Thermococcus litoralis MBP
MKIEEGKIVF AVGGAPNEIE YWKGVIAEFE

THE INDUDUCA	D CLOCK CHOOK TATE	_		
MKIEEGKIVF	AVGGAPNEIE	YWKGVIAEFE	KKYPGVTVEL	KRQATDTEQR
RIDLVNALRG	KSSDPDVFLM	DVAWLGQFIA	SGWLEPLDDY	VQKDNYDLSV
FFOSVINIAD	KOGGKLYALP	VYIDAGLLYY	RKDLLEKYGY	SKPPETWQEL
VEMAOKTOSG	ERETNPNFWG	FVWQGKQYEG	LVCDFVEYVY	SNGGSLGEFK
DCKWVPTLNK	PENVEALOFM	VDLIHKYKIS	PPNTYTEMTE	EPVRLMFQQG
NAAFERNWPY	AWGLHNADDS	PVKGKVGVAP	LPHFPGHKSA	ATLGGWHIGI
		SVQKGFAMNL		
HIKELBAVEE	NAVPRPTVPY	YPQLSEIIQK		
KEAEELVKQY				- ~
VENEEDANAT	OIL			

#### **FIG. 43D**

Thermatoga n	naritime MBP			
MKTEOTKLTI	WSSEKOVDIL	OKLGEEFKAK	YGIPVEVQYV	DFGSIKSKFL
TAAPOGOGAD	IIVGAHDWVG	ELAVNGLIEP	IPNFSDLKNF	YDTALKAFSY
GGKLYGVPYA	MEAVALIYNK	DYVDSVPKTM	DELIEKAKQI	DEEYGGEVRG
FTYDVANEYF	SAPFILGYGG	YVFKETPQGL	DVTDIGLANE	GAVKGAKLIK
RMTDEGVLTP	GDNYGTMDSM	FKEGLAAMII	NGLWAIKSYK	DAGINYGVAP
<b>IPELEPGVPA</b>	KPFVGVQGFM	INAKSPNKVI	AMEFLTNFIA	RKETMYKIYL
ADPRLPARKD	VLELVKDNPD	VVAFTQSASM	GTPMPNVPEM	APVWSAMGDA
LSIIINGQAS	VEDALKEAVD	KIKAQIEK		

#### FIG. 43E SUBSTITUTE SHEET (RULE 26)

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## Wibrio cholerae MBP MKIEEGQLTI WINGDKGYNG LAEVGKKFEA DTGIKVTVAH PDALQDKFPQ TAATGDGPDI VFWAHDRFGG YAEAGLLVEI KPSAKIQEGI VDFAWDAVKY NGKIIGYPIA VESLSLIYNK DLVPNPPKSW EEVAELDAKL KKEGKSAIMW NLKEPYFTWP LMAADGGYAF KYGVDGYDVK DAGINNKGVK DAMNFVKGLV DKGVISPDMD YSVSESAFNQ GNTAMTINGP WSWGNIEKSG INYGVTTLPK FNGQASKPFV GVLTAGISTA SPNKDLAVEF IENYLLTNDG LRMVNNDKPL GAVALNSFQR ELDADARIAA TMDNAMNGEI MPNIPQMNAF WSSAKNAIIN

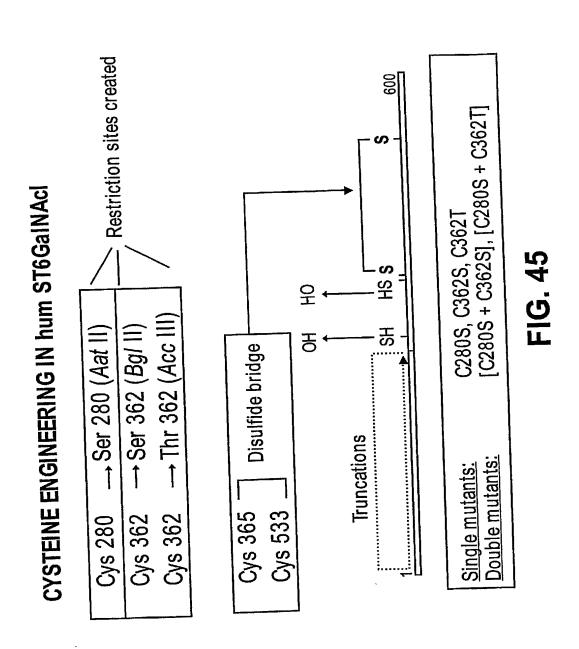
**FIG. 43F** 

IVDGRQTVDA ALADAEKQMT KP

53/54 50 HSGALNAT1.pep (1)MRKFAYCKVVLATSLIWVLLDMFLLLYFS----ECNKC HSGALNAT2.pep (1)MRRRS--RMLLCFAFLWVLGIAYYMYSGGGSALAGGAGGGAGRKEDWNEI (1)MRK A KMLL A IWVL Consensus F L D N 51 100 HSGALNAT1.pep (35) DEKKERGLPAGDVLEPVQKPHEGP-G----EMGKPVVIPKEDQEKMKEM HSGALNAT2.pep (49) DPIKKKDLHHSNGEEKAQSMETLPPGKVRWPDFNQEAYVGGTMVRSGODP (51)D KKL Consensus E Q PGD 101 150 (79) FKINQFNLMASEMIALNRSLPDVRLEGCKIKVYPDNLPITSVVIVFHNEA HSGALNAT1.pep (99) YARNKFNQVESDKLRMDRAIPDTRHDQCQRKQWRVDLPATSVVITFHNEA HSGALNAT2.pep Consensus (101)F N FN M SD I L RAIPD R D C K W LP TSVVI FHNEA 151 200 HSGALNAT1.pep (129)WSTLLRTVHSVINRSPRHMIEEIVLVDDASERDFLKRPLESYVKKLKVPV HSGAINAT2.pep (149)RSALLRTVVSVLKKSPPHLIKEIILVDDYSN----DPEDGALLGKIEKV Consensus (151) S LLRTV SVI KSP HLI EIILVDD S PD L V 201 250 HSGALNAT1.pep (179) HVIRMEQRSGLIRARLKGAAVSKGQVITFLDAHCECTVGWLEPLLARIKH HSGALNAT2.pep (194) RVLRNDRREGLMRSRVRGADAAQAKVLTFLDSHCECNEHWLEPLLERVAE Consensus (201) VIR D R GLIRARLKGA A A VITFLDAHCEC HSGALNAT1.pep (229) DRRTVVCPIIDVISDDTFEYMAGSDMTYGGFNWKLNFRWYPVPQREMDRR HSGALNAT2.pep (244) DRTRVVSPIIDVINMDNFQYVGASADLKGGFDWNLVFKWDYMTPEQRRSR Consensus (251)DR VV PIIDVI D F YMAAS GGF W L FKW M R 301 350 HSGALNAT1.pep (279)KGDRTLPVRTPIMAGGLFSIDRDYFQEIGTYDAGMDIWGGENLEISFRIW HSGALNAT2.pep (294)QGNPVAPIKTPMIAGGLFVMDKFYFEELGKYDMMDVWGGENLEISFRVW PIKTP IAGGLF IDK YF EIG YD MDIWGGENLEISFRIW Consensus (301) G 351. HSGALNAT1.pep (329)QCGGTLEIVTCSHVGHVFRKATPYTFPGGTGQIINKNNRRLAEVWMDEFK HSGALNAT2.pep (344)QCGGSLEIIPCSRVGHVFRKQHPYTFPGGSGTVFARNTRRAAEVWMDEYK Consensus (351)QCGGSLEII CS VGHVFRK PYTFPGGSG I KN RR AEVWMDEFK 401 HSGALNAT1.pep (379)NFFYIISPGVTKVDYGDISSRVGLRHKLQCKPFSWYLENIYPDSQIPRHY HSGALNAT2.pep (394)NFYYAAVPSARNVPYGNIQSRLELRKKLSCKPFKWYLENVYPELRVPDHO V YG I SRL LR KL CKPF WYLENIYPD Consensus (401)NFFY IP H 451 500 HSGALNAT1.pep (429) FSLGEIRNVETNQCLDNMARKENEKVGIFNCHGMGGNQVFSYTANKEIRT HSGALNAT2.pep (444) DIAFGALQQGTN-CLDTLGHFADGVVGVYECHNAGGNQEWALTKEKSVKH Consensus (451) N TN CLD LA VGIF CH GGNQ FA T K IK 501 HSGALNAT1.pep (479) DDLCLDVSKLN--GPVTMLKCHHLKGNQLWEYDPVKLTLQHVNSNQCLDK HSGAINAT2.pep (493)MDLCLTVVDRAPGSLIKLQGCRENDSRQKWEQIEGNSKLRHVGSNLCLDS Consensus (501) DLCL V IL C QWE L HV SN CLD 551 584 HSGALNAT1.pep (527) ATEEDSQVPSIRDCNGSRSQQWLLRNVTLPEIF-HSGALNAT2.pep (543) RTAK-SGGLSVEVCGPALSQOWKFTLNLQQ----Consensus (551) T S SI C A SQQW

### FIG. 44 SUBSTITUTE SHEET (RULE 26)

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